PATENT COOPERATION TREATY

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From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB2005/000795	23.03.2005	26.03.2004

International Patent Classification (IPC) or both national classification and IPC
G01N21/64, G01N33/52, C07K14/435

Applicant
RIZZUTO, Rosario

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 6-43, 47-50, and 1-5, 44-46 (partially)

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 6-43, 47-50, and 1-5, 44-46 (partially)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. Invention 1: Claims 1-5 and 44-46 (all partial).

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-5, 44-46
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-5, 44-46
Industrial applicability (IA)	Yes:	Claims	1-5, 44-46
	No:	Claims	

2. Citations and explanations

   see separate sheet

III No opinion.

The international search report (ISR) was not established for claims 6-43, 47-50, and 1-5, 44-46 (partially). Consequently, no opinion will be formulated with respect to the subject matter of these claims.

IV Lack of unity of invention.

Art. 17(3)(a) and Rule 13 PCT require that the international application shall relate to one invention only or to a group of inventions so linked as to form a single general novel and inventive concept, which is defined by special technical features that make a contribution over the cited prior art (cf. Guidelines 10.01-10.02).

The problem to be solved with the present application is the provision of a method for the screening of molecules that can modify intracellular parameters, such as second messengers.

Methods for the detection of alterations in the concentrations of second messengers are abundantly known, and comprise for instance the use of GFP- or aequorin-chimera (see for instance: Chiesa et al. (2001) for review).

The solution of the applicant is characterised by an indirect measurement of the modification of the intracellular parameter by conversion thereof into a corresponding modification of the Ca^{2+} concentration, which can then be measured.
Three solutions can be found in claim 1:
A) The intracellular second messenger itself induces the release of intracellular Ca^{2+} proportional to its own variation,
B) The provision of recombinant chimeric protein probes comprising a Ca^{2+}-photo-protein and an effector protein that responds to the change in the intracellular parameter, and
C) The provision of a recombinant chimeric receptor, that is modified in such a away that it influences the intracellular Ca^{2+} concentration instead of the parameter to be determined.

In addition, the applicant provides probes for use in the screening methods.

The concept that links the subject matter of independent claims 1, 31 and 44 is the probes of claim 31. These probes lack novelty (see: Brini et al. (1994), Chiesa et al. (2001), Torfs et al. (2002)). In addition, the use of these probes is not restricted to any of the methods, as is evidenced by Brini et al. and Chiesa et al.. These probes are therefore not linked to the methods by a general novel and inventive concept.

The concept that links groups A, B and C as defined above is a method of screening for compounds that modify an intracellular parameter comprising converting said alteration into a proportional variation of the Ca^{2+} ion.

This concept lacks novelty over Torfs et al. (2002) and an inventive step in view of Brini et al. (1994).

Torfs et al. (2002) discloses a screening assay for agonists of the tachykinin-related peptide receptor STKR, using a recombinant chimeric Ca^{2+} sensitive aequorin as a photo-protein that additionally comprises a mitochondrial signal sequence. The agonist induces an increased level of intracellular IP3, which in its turn is converted into an increased level of Ca^{2+}, which is measured by the photo-protein.

Brini et al. (1994) discloses the use of a recombinant chimeric shuttle protein comprising a part of the glucocorticoid hormone receptor and the Ca^{2+} sensitive photo-protein aequorin (nu/cyt-AEQ) for the determination of the presence of a stimulating molecule (cf. Fig. 1). This chimeric proteins responds to a change in the intracellular InsP3 concentration with a translocation from the cytosol to the nucleus, and thus reports upon activation with said growth hormone the Ca^{2+} concentration in the nucleus, whereas in rest it reports it reports the cytosolic Ca^{2+} concentration.
It is stated: "*Upon stimulation with the InsP3-generating agonist histamine, a sharp increase ... When the agonist was removed, light emission readily returned to resting values.*" (cf. D1, p. 264, r. col). This procedure is identical to the one disclosed for the chimeric probes for use in solution B (cf. p. 10, l. 15 - p. 11, l. 13 of the description).

The groups described above thus represent three different inventions that are not linked via a novel and inventive concept contrary to the provisions of Rule 13 PCT.

In addition the probes of claim 31 need to be divided into two groups:
D) Probes for use in the method of group B, and
E) Probes for use in the methods of groups A and C.

Probes falling within the scope of both groups have been disclosed (see: Brini et al., Chiesa et al. and Torfs et al.). These probes are therefore not linked by a novel and inventive concept.

The international search report has been established for invention 1 as defined above, because it is the first mentioned in the claims (eg. claim 4). Enlarging the scope of the search report to other inventions would require a considerable additional effort.

This written opinion will therefore only formulate an opinion on the subject matter of invention 1.

It is noted that although some of the documents are also cited against claims that are not part of the searched invention this should not be taken as an indication that other inventions were searched, but merely serves as a clarification for the objection for a lack of unity of invention.

V Reasoned Statement.

Subject matter of the present application.

The provision of method for screening for compounds capable of altering an intracellular parameter, comprising converting this alteration into a proportional variation in the intracellular Ca^{2+} concentration, and measuring this Ca^{2+} variation using a recombinant chimeric Ca^{2+} sensitive photo-protein that comprises a Ca^{2+} sensitive photo-protein and either a cellular effector or a signal sequence.

Cited prior art documents (Rule 64(1) PCT).

D1: TORFS ET AL. (2002) INVERT. NEUROSCI. 4, 119-124.
D2: WO 03/082904 A.

D3: LE POUL ET AL. (2002) J. BIOMOL. SCREENING 7, 57-65.
D4: BRINI ET AL. (1994) CELL CALCIUM 16, 259-268.
D5: CHIESA ET AL. (2001) BIOCHEM. J. 355, 1-12.
D6: US 6566083 B1.

Novelty (Art. 33(2) PCT).

D1 discloses a screening assay for agonists and antagonists of the tachykinin-related peptide receptor STKR, using a recombinant chimeric Ca^{2+} sensitive aequorin as a photo-protein that additionally comprises a mitochondrial signal sequence (cf. Fig. 1, Fig. 3, Materials and methods). The agonist induces an increased level of intracellular IP3, which in its turn is converted into an increased level of Ca^{2+}, which is measured by the photo-protein. It is concluded: "*The present study shows that agonists as well as antagonists can be studied by analysing bioluminescent responses generated in an insect cell line expressing both apoaequorin and STKR, and insect tachykinin-related GPCR. ... Since measurement can be easily performed in a microplate luminometer, this functional, insect-cell-based, receptor assay system can be utilised as a powerful tool for screening natural or synthetic compound libraries resulting in the discovery of new receptor ligands.*" (cf. D1, General conclusion, p. 123, l. col.).

D1 is prejudicial to the novelty of the subject matter of claims 1-5, 21, 31, 32 and 43-46.

D2 discloses a comparable screening assay using aequorin derivatives with enhanced glowing characteristics (cf. Fig. 1). D2 intends to use recombinant chimeric proteins having the modified aequorin and a cell compartmentalization domain or receptor ligand for targeting of the modified aequorin to specific cell compartments or receptors (cf. p. 16, l. 1-18).

D2 is prejudicial to the novelty of the subject matter of claims 1-5, 21, 31, 32 and 43-46.

D3 again discloses a comparable screening assay for compounds that interact with a receptor of the GPCR family (cf. aequorin assays for details of the screening method). D3 is prejudicial to the novelty of the subject matter of 1-5, 21, 31, 32 and 43-46.

D4 discloses the use of a recombinant chimeric shuttle protein comprising a part of the glucocorticoid hormone receptor and the Ca^{2+} sensitive photo-protein aequorin (nu/cyt-AEQ) for the determination of the presence of a stimulating molecule (cf. Fig. 1). This chimeric proteins responds to a change in the intracelluiar InsP3 concentration with a translocation from the cytosol to the nucleus, and thus reports upon activation with said growth hormone the Ca^{2+} concentration in the nucleus, whereas in rest it reports it reports the cytosolic Ca^{2+} concentration.

It is stated: "*Upon stimulation with the InsP3-generating agonist histamine, a sharp*

increase ... When the agonist was removed, light emission readily returned to resting values." (cf. D1, p. 264, r. col). This procedure is identical to the one disclosed for the chimeric probes for use in solution B (cf. p. 10, l. 15 - p. 11, l. 13 of the description).
D4 is prejudicial to the novelty of the subject matter of claims 31-34 and 42.

D5 is a review on the use of recombinant aequorin to measure cell signalling. In Fig. 2 several different aequorin derivatives are shown that all comprise a compertamentalization domain.
D5 is prejudicial to the novelty of the subject matter of claims 31, 32 and 43.

D6 discloses screening assays for compounds that affect intracellular events based on the GFP protein. The GFP proteins do not require a prosthetic group. D6 is therefore not prejudicial to the novelty of claim 1. Moreover the intracellular event is not converted into a Ca^{2+} signal.

To summarize: the subject matter of claims 1-5 and 44-46 lacks novelty over the cited prior art documents (Art. 33(2) PCT).

Inventive step (Art. 33(3) PCT).
Because claims 1-5 and 44-46 lack novelty they also lack an inventive step.

Industrial applicability (Art. 33(4) PCT).
The subject matter of claims 1-5 and 44-46, in so far as they relate to invention 1, meets the requirement of industrial applicability.